GROWTH REGULATORS AND THE FLOWERING OF EVERGREEN AZALEAS (*RHODODENDRON* CV.)

by
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Title of Thesis: Growth Regulators and the Flowering of Evergreen Azaleas (Rhododendron cv.)

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ABSTRACT

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Spraying experiments were conducted in 1958 and 1959 to find the effectiveness of certain growth regulators upon multiple flower bud formation and rate of flower bud development of evergreen azaleas. Paper chromatograms of extracts of vegetative buds and of flower buds treated with 37°F. storage and potassium gibberellate (GAk) sprays, were tested with a wheat coleoptile bioassay in 1959.

Foliar sprays of 2, 3, 5-triiodobenzoic acid (TIBA), an antiauxin, showed evidence of inhibiting multiple flower bud formation, and a foliar spray of 2,200 ppm indoleacetic acid (IAA) tended to promote multiple flower bud formation. The time of spraying in relation to the time of floral initiation apparently is important if growth regulators are to influence multiple flower bud formation.

The rate of flower bud development was increased by two weeks of 37°F. storage and either two sprays of 200 ppm TIBA or single sprays of 160, 400 or 1,000 ppm TIBA, or by three weeks of 45°F. storage and a single spray of 1,000 ppm IAA. Rate of flower bud development was increased by

two sprays of 200 ppm TIBA and one spray of 1,000 ppm gibberellic acid (GA).

Flower bud dormancy was removed by foliar sprays of 900 ppm GAk with no cold storage or two weeks of 37°F. Four weeks of 37°F. storage was effective without GAk and six weeks of 37°F. storage gave no increase over four weeks of storage. Concentrations of GAk lower than 900 ppm were ineffective. GAk was effective whether applied before or after two weeks of 37°F. storage.

Naphthalene acetic acid in concentrations of 9 ppm or greater inhibited the rate of flower bud development.

Apical dominance was removed by 800 ppm or more of TIBA.

The wheat coleoptile bioassay indicated that a growth inhibitor in the flower buds was removed by three or more weeks of 37°F. storage and three sprays of 1,000 ppm GAk. The promoter was not in vegetative buds and could not be considered to be IAA, GA or GAk.

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INTRODUCTION

In order for flower buds to develop on the azalea they require a period of a temperature of 40 to 55° F. (6, 43, 73, 75, 81, 83). These temperatures are less than the 65° F. temperature recommended for flower initiation and the 60 to 65° F. temperature recommended for forcing (45, 74). Low temperatures are needed so that the flower buds will force evenly and in a minimum of time. Certain varieties, particularly the Belgian or Indica varieties of the trade, have been forced for late December and January flowering. During the past few years some florists have developed a year-round azalea flowering program, and thus the so-called cold period requirement or flower bud dormancy now has greater significance.

One of the chief drawbacks to such a year-round program has been the need for controlled temperature storage rooms and their relative high cost. It was thought that applications of a chemical to bypass the cold period might be more economical. Boodley and Mastalerz (10) and Martin, Wiggans and Payne (62) used gibberellic acid to remove flower bud dormancy, but the concentrations they used were so great that the process was not economical. Thus, it was felt that use of a shorter cold period (less than six weeks) and lower gibberellin concentrations (less than 1,000 ppm) might have an effect similar to higher

gibberellin concentrations or four to six weeks cold, and might be commercially feasible.

Blommaert (8), Hendershott and Bailey (36), Nitsch (67) and Phillips and Wareing (70) have reported that in the removal of bud dormancy with low temperature or long day photoperiods that either a growth inhibitor decreases in concentration or a growth promoter increases. This growth promotion or inhibition is measured by a bioassay utilizing the increase or decrease in growth of sections of wheat or oat coleoptiles. It was thought that possibly there might be a similar relationship involved in the removal of the flower bud dormancy of the azalea.

Azaleas may have one or more flowers in each flower bud. It was thought that if growth regulators could be found to affect multiple flower bud formation, then some information on the mechanism of floral initiation in the azalea might be obtained. Only nitrogen fertilization has been reported as affecting multiple flower bud formation in the azalea (45, 82).

The objectives of this study were:

(1) To measure the effectiveness of growth regulating chemicals and varying periods of cold in the removal of flower bud dormancy of the azalea.

(2) To measure the effect of low temperatures and gibberellin applications upon the levels of endogenous growth substances within azalea flower buds.

(3) To measure the effectiveness of growth regulating chemicals upon multiple flower bud formation of the azalea.

REVIEW OF LITERATURE

In 1940, Skinner (83) used temperatures of 35 to 50° F. to hasten the flower bud development of azaleas and rhododendrons. In 1943, Post (73) found that the azalea variety Coral Bells required four weeks of 40-41°F. storage and the variety Triumph required six weeks of 40-41° F. storage. He also found that Coral Bells flowered in four weeks at 60° F. following cold storage, and that Triumph flowered in six or seven weeks at 60° F. following cold storage. Kiplinger (43) reported that shading the plants with black cloth and giving them 10 hour days for one month prior to cold storage gave a further stimulus to early flowering. Post (74) stated that flower buds grow to a certain size at high temperature and then must have some temperature treatment below 60° F. so that they may be forced at high temperature in a minimum period of time.

Doorenbos (25) has reported that flower buds of the rhododendron variety Cunningham's White could be forced into flower as early as September by continuous light. Later Doorenbos (26) reported similar results for Rhododendron catawbiense album and for azalea seedlings of a Rhododendron japonicum x Rhododendron molle cross. Langhans (49) found that the forcing period of azaleas could be reduced if the plants were exposed to incandescent or fluorescent lights

24 hours per day, but that flower color was less intense. Kiplinger (43) stated that lights were needed in storage to keep leaves on the plants. Post (74) indicated that the reason that lights were not needed for a storage temperature of 40° F. was that the respiration rate was lower and the food supply was not depleted.

In 1956, Lang (47) found that when plants of the biennial form of Hyoscyamus niger were kept at a temperature above the maximum for floral initiation and were treated with gibberellin, elongation and flowering followed under long day conditions. Under short day conditions, the elongation was less marked and it was not followed by flowering. The control plants remained as vegetative rosettes. In a later paper, Lang (48) reported that gibberellin could act as a cold substitute for flowering in carrots, parsley and turnips. In the same year Barton (4) reported that gibberellic acid promoted the growth of physiologic dwarfs from nonafterripened embryos of Malus arnoldiana, and that the chemical was effective because it induced the extension of internodes. Later, workers found that gibberellin was a substitute for the cold temperature requirement of many species and physiological conditions. Barton and Chandler (5) found that gibberellic acid could replace the low temperature requirement for removal of epicotyl dormancy of the tree peony. Carr, McComb and Osborne (17) found a similar response to gibberellic acid in Centaurium minus to the response that Lang had found in Hyoscyamus niger.

Rosette plants, given gibberellic acid and long days would elongate and flower, but under short days the rosette plants would elongate and not form flower buds. Donoho and Walker (24) reported that gibberellic acid could break the rest period and reduce the chilling requirement of the Elberta peach. Chailakhyan (18) found that winter rape and seedlings of biennials bolted when denied low temperatures and were treated with gibberellin. Lindstrom, Wittwer and Bukovac (56) reported that gibberellin hastened the flowering of stocks, larkspur, petunia, English daisy, China aster, gerbera and foxglove. The foxglove is a cold requiring biennial and Post (74) stated that stocks require low temperatures for flower bud formation. Harada and Nitsch (33) found that gibberellin could induce three Japanese varieties of Chrysanthemum morifolium to flower. These varieties were insensitive to photoperiod, but required a cold period for floral initiation. Boodley and Mastalerz (11) and Martin, Wiggans and Payne (62) reported that gibberellic acid could be used on azaleas instead of low temperature to remove flower bud dormancy. Their concentrations were high, their applications were frequent and no interactions with low temperature were reported.

In a 1957 review of research on the gibberellins Stowe and Yamaki (86) reported that gibberellin could induce:

1) Stem elongation through cell elongation;

2) Growth of dwarf plants;

3) Leaf expansion;

4) Reduction of root growth in comparison to top growth;
5) Chlorosis;
6) Flowering by substituting for long days in long day plants given short days;
7) Parthenocarpy of fruits;
8) Reduction of time for seed germination;
9) Reversal of red light inhibition of growth;
10) The following biochemical effects:
    a. Decrease in starch levels;
    b. Increase in oxygen consumption;
    c. Greater activity of pectin methyl esterase, phosphatase and B-amylase;
    d. Increase of growth response with L-tryptophane;
11) Lack of epinasty of tomato leaf petioles (auxins would induce such epinasty).

Considerable research has been undertaken since the review of Stowe and Yamaki was published. Sachs and Lang (79) found that gibberellin induced mitosis in the subapical region of the biennial form of Hyoscyamus niger. Gundersen (31) found that gibberellin could promote stem elongation in a begonia hybrid partly by extension of cell walls, and partly by cell division, and that the chemical could accelerate cambial activity. Leivonen (50) reported that gibberellin could induce cell division in the roots of Narcissus tazetta but that indole acetic acid could also induce such mitosis.

Brian, Petty and Richmond (15) found that gibberellic acid applications can cause a delay in the development of autumn foliage color, a delay in leaf abscission, or a renewal of shoot growth--all of them typical long day responses. Bukovac and Davidson (16) reported that gibberellin could substitute for long days for shoot extension of Weigela. Lockhart and Bonner (61) found that when camellia buds were physiologically able to resume growth, that gibberellic acid could replace the long day requirement for initiation of new growth.

Liverman and Johnson (57) found that the arrested fruit growth of Marglobe tomatoes induced by high light intensities and temperatures could be reversed by gibberellin, red light or cool temperatures. Kahn, Goss and Smith (40) reported that gibberellin could act as a substitute for red light in promoting the germination of light-sensitive lettuce seed. Vlitos and Meudt (91) found that gibberellic acid could overcome the inhibitory effect of red, blue or green light upon the elongation of stems of Alaska pea seedlings. Lockhart (59, 60) reported that Phaseolus vulgaris would elongate if given red light and gibberellin, and that the inhibition of stem growth of Pisum sativum, Phaseolus vulgaris, Cucumis sativus, Cucurbita pepo and Helianthus annus caused by red or blue radiation could be inhibited by gibberellic acid.

Pilet and Wurgler (72) reported a decrease in auxin oxidase activity following gibberellin treatments. Galston

and Warburg (30) suggested that the additive response to gibberellin and auxin might involve an auxin-sparing mechanism and McCune and Galston (62) found that gibberellin could decrease the peroxidase activity of dwarf corn. Bergqvist, Stensgaard and Nielsen (7) reported that gibberellic acid stimulated an increase in glutamic-oxaloacetic transaminase activity, and in glutamic-pyruvic transaminase activity. Linck and Suda (55) found that gibberellin treatments could produce an increase in phosphorous absorption.

Schroeder and Spector (80) found that indoleacetic acid would stimulate the callus formation response of gibberellic acid. Brian and Hemming (13) stated that gibberellic acid would induce the extension of green pea-stem sections only if an auxin was present. Wareing (93) reported that gibberellic acid stimulated cambial division, while indole acetic acid caused vacuolation and lignification of the resulting cambial derivatives. Kato (41) found that gibberellic acid acted additively with concentration ranges of indole acetic acid and naphthalene acetic acid where shoot growth was promoted, and reversed the inhibition of shoot growth by higher concentration of indole acetic acid and naphthalene acetic acid. Clor, Currier and Stocking (20) reported a synergistic response between 2, 4-dichlorophenoxyacetic acid and gibberellic acid in the promotion of shoot elongation of cotton seedlings. Weijer (94) reported that smaller amounts of indole acetic acid enhanced the stem

elongation of Impatiens by gibberellic acid but that higher concentrations of indoleacetic acid would inhibit such a response. He felt that higher concentrations of auxin stimulate the production of auxin inhibitor.

Other responses to gibberellin include the induction of male sterility in inbred maize (65) and the inhibition of branching in sweet peas (14).

Phinney, West, Ritzel and Neely (71) and Radley (76, 77) have reported the extraction of gibberellin-like substances from plants. These substances induce gibberellin-like physiological responses but do not fluoresce with sulfuric acid as do the gibberellins.

Many workers have found that external stimuli have affected levels of endogenous growth substances within plants. Vlitos and Meudt (90) found an increase in the levels of 3-indolepyruvic acid and indoleacetic acid in the leaves of Biloxi soybean and Maryland Mammoth tobacco plants grown under photoinductive daylengths. Vlitos and Meudt warned that possibly these compounds had no effect upon floral initiation, but that their synthesis could be independently influenced by daylength. Khudairi and Bonde (42) mentioned that a growth inhibitor in Xanthium pennsylvanicum leaves decreased as short day exposure was increased. Cooke (22) found a decrease in auxin level accompanying the appearance of floral primordia in Xanthium, an increase in auxin content as the plant was moved from long days to short days, and an increased auxin content in plants grown under long days.

Cooke could not associate a drop in auxin content with flowering. Phillips and Wareing (70) found that an inhibitor increased in sycamore buds in early winter and again in late summer, and that the inhibitor was at a minimum in May and June. Harada and Nitsch (32) reported that there was an increase in growth promoters in the stem tips of the long day plant Rudbeckia speciosa during long days.

Cold temperature has been reported as having an effect upon the endogenous growth substances within buds. Blommaert (8), and Hendershott and Bailey (36) reported the presence of a growth inhibitor within peach flower buds, the level of the inhibitor decreasing as the buds lose their dormancy. Hendershott and Walker (37) tentatively identified this inhibitor as naringenin. Hendershott and Walker (38) found three and Blommaert (8) four growth promoting substances, but in each case these were at their maximum levels during the breaking of dormancy. Harada and Nitsch (32) reported an increase in two growth promoters and the reduction of a third in the cold requiring Japanese chrysanthemum variety Shuokan when it is exposed to cold.

Other workers have found that chemicals can affect the levels of endogenous growth substances within plants. Hemberg (35) found that ethylene chlorohydrin would cause inhibiting substances in resting potatoes to disappear as rest was broken. Audus and Thresh (3) reported that 2, 3, 5-triiodobenzoic acid reduced the levels of endogenous indoleacetic acid in pea roots but that 2, 4-dichlorophenonyacetic

acid did not have a similar effect because it is an auxin in its own right. Nitsch (67) reported that in sumac buds gibberellic acid caused an increase of a growth promoter that occurred in the same chromatogram region as indole-acetic acid. Phillips, Vlitos and Cutler (69) found that Alaska pea seedlings sprayed with gibberellic acid had a 180% increase in endogenous growth promoters after 24 hours.

Little research has been done on floral initiation in the azalea. Skinner (83) showed that a temperature of 75-80° F. would stimulate flower initiation of azaleas compared to a temperature range of 50-55° F. Borthwick, Parker and Rappleye (12) and Kiplinger (44) found that daylength had little influence upon floral initiation. Kiplinger and Bresser (46) and Shanks, Link and Preston (82) have shown the effectiveness of nitrogen fertilization in increasing multiple flower bud formation.

Leopold (51) lists three different types of auxin application employed to affect flowering:

1) Spraying auxin on the foliage of vegetative plants to induce flowering;
2) Spraying auxin at the time of floral initiation or shortly thereafter to increase or decrease flowering;
3) Soaking seeds in an auxin solution.

In 1935, Hitchcock and Zimmerman (39) found that tobacco flowering could be hastened by soil applications of indolebutyric, indolepropionic and phenylacetic acids. In

1942, Clark and Kerns (19) reported that the pineapple could be induced to flower by applications of naphthalene acetic acid. Later, Leopold and Thimann (54) found that low concentrations of naphthalene acetic acid could promote the flowering of barley given photoinductive long days. These workers also reported that all auxin concentrations inhibited the flowering of teosinte when it received photoinductive short days. Bonner and Thurlow (9) reported that foliar sprays of naphthalene acetic acid or indoleacetic acid could suppress floral initiation of the cocklebur if applied during the photoinductive period. Leopold and Guernsey (52) reported that soaking seeds of barley, teosinte, soybean, oats and corn in naphthalene acetic acid and following this treatment with cold could promote flowering. Later, Leopold and Guernsey (53) reported a similar response by the Alaska pea. DeZeeuw and Leopold (23) suggested that because auxin treatments promoted flowering in otherwise juvenile plants, that the completion of the juvenile phase might impart the accumulation of an auxin level at the apical meristem sufficient to bring about a condition receptive to cold. Liverman and Lang (58) found that two long day plants, Hyoscyamus niger and Silene armeria, could be induced to flower by the application of indole acetic acid to plants grown under threshold conditions.

2, 3, 5-triiodobenzoic acid, which can act as an anti-auxin, has also affected flowering. Zimmerman and Hitchcock (95) found that it could induce tomato flower cluster

production from axillary buds which normally produce leafy shoots, and from the main shoot of the plant, which usually terminates in the shoot-producing bud. Galston (29) found that 2, 3, 5-triiodobenzoic acid could augment the flowering response of the soybean due to short days.

MATERIALS AND METHODS

Analyses of variance were computed for the data of all experiments. Differences between treatment means were tested with Duncan's multiple range test (26, 27) or the least significant difference (21), using the 5% level of significance in either case. All spraying experiment data were percentages, and these data were transformed to arc sin values (84) before any calculations were made. Glucose was used in the sprays because Rohrbaugh and Rice (78) found that sugars would aid in the translocation of 2, 4-dichlorophenoxyacetic acid (2, 4-D). Boric acid was added because Mitchell, Dugger and Gauch (64) reported that boron had a stimulatory effect upon 2, 4-D translocation. It was felt that glucose and boric acid might stimulate the translocation of growth regulators other than 2, 4-D. Techniques described by Anderson and Houseman (1) for fitting equations by the method of orthogonal polynomials were used to describe possible trends in some of the spraying experiments.

A. SPRAYING EXPERIMENTS.

Experiment 1. This experiment was undertaken during the summer of 1958 with the goal of testing the effect of 3-indoleacetic acid (IAA) and 2, 3, 5-triiodobenzoic acid (TIBA) upon multiple flower bud formation. Plants of the varieties Vervaeneana, Hexe, Dorothy Gish and Rose Greeley

were used. All of these plants had been topped May 1, 1958. The plants were sprayed with 0, 50, 150 and 450 ppm of each chemical dissolved in a minimum of alcohol first and next in a solution consisting of 500 ppm glucose, 500 ppm boric acid and 0.1% Tween 20. Sprays were applied June 20 or July 11 and upon both June 20 and July 11. Plants of each variety were arranged in a completely randomized design, with eight plants in each treatment. Plants remained in the greenhouse until September 11 when four plants from each treatment and each variety were placed in 35° storage for six weeks. After storage these plants were forced at 65° F. night temperature. On September 25, four plants from each treatment of the Vervaeneana, Hexe and Dorothy Gish varieties were placed in a heated cold frame. Vervaeneana and Hexe plants were brought back into the greenhouse March 5, 1959, and were forced into flower at 65° night temperature. Dorothy Gish plants were allowed to flower in the cold frame. Data was collected as the number of flowers per flower bud. In the case of Vervaeneana the data was analyzed as the arc sin transformation of the percentage that single flower buds were of all flower buds. In the case of Hexe and Rose Greeley the data were analyzed as the arc sin transformation of the percentage that one-and two-flower buds were of total flower buds. The Dorothy Gish data were analyzed as the arc sin transformation of the percentage that four-flower buds were of total flower buds. All treatments were compared with the check using the LSD test.

Experiment 2. Plants of the variety Vervaeneana were sprayed on July 3, 1958 with 0, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200 and 2400 ppm of IAA and TIBA. There were three plants per treatment, in a completely randomized design. These plants went into cold storage September 11, 1958 for six weeks and were forced into flower at 65° F. night temperature. Data were collected and analyzed in the same way from these plants as from the Vervaeneana plants of experiment 1.

Experiment 3. This spraying experiment was undertaken during the fall of 1958. Plants of the variety Triumph were received in flats June 10, 1958 from a commercial propagator. These plants were potted into 4" clay azalea pots and were grown until the fall. At that time the plants were treated with growth regulators and subjected to different cold temperature treatments. Only single applications of the chemicals were given to the plants. The aim of this preliminary experiment was to find a growth regulating chemical which would advance flower bud development when the cold period was of a threshold amount. The chemicals used were 3-indoleacetic acid (IAA), naphthalene acetic acid (NAA), 2, 3, 5-triiodobenzoic acid (TIBA) and the potassium salt of gibberellic acid (GAk).[1] Concentrations of chemicals were

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[1]The potassium gibberellate used in this and other experiments was kindly donated by Merck & Co., Inc., Rahway, N. J.

64, 160, 400 and 1,000 ppm. The chemicals were dissolved into water containing 500 ppm glucose and 500 ppm boric acid. IAA was dissolved in the glucose-boric acid solution with 35 ml. 95% ethanol per litre. TIBA required 200 ml. 95% ethanol per litre, NAA required 100 ml. 95% ethanol per litre and GAk required 20 ml. 95% ethanol per litre. The highest concentration was made up using 1g. of chemical and the above mentioned quantities of 95% ethanol. The other concentrations were produced by dilution of next highest concentration with glucose-boric acid solution. Each concentration of each chemical was applied to plants that had received 0, 1, 2, 3, 4 and 5 weeks of cold storage. Plants that had received no growth regulators, but were sprayed with the glucose-boric acid solution were treated with 0, 1, 2, 3, 4 and 5 weeks of cold and served as checks. All of the solutions contained 0.1% Tween 20. 600 ml. of each solution was sprayed on the four plants or replications of each treatment. A randomized block design was used.

Plants were placed in a 45° F. storage October 10, and were illuminated by incandescent and fluorescent lights for 8 hours each day to prevent leaf drop (74). Plants were removed from storage October 17, October 24, October 31, November 7 and November 14. Plants received sprays on the day following removal from storage. Before and after storage the plants were in a greenhouse maintained at 60° F. at night. As anthesis occurred, the flowers were removed from each plant and counted. The first data were taken December

19, 1958 and the final data were taken on February 23, 1959. The data were analyzed as the arc sin transformation of the percentage that flowers open eight weeks after the final treatments were of total flower buds. Differences between means in this and succeeding experiments were tested by Duncan's multiple range test.

Experiment 4. Plants of the variety Triumph similar to those employed in experiment 3 were used in this experiment. 10 lambda of 1,000 ppm solutions of IAA, TIBA, NAA and GAk as well as a check solution were added with a micropipette directly to individual flower buds. All solutions contained 95% ethanol (50%), 250 ppm glucose, 250 ppm boric acid and were 0.1% Tween 20. The buds of plants receiving no cold storage and two weeks 45° F. storage received 10, 20, 30, 40 and 50 lambda of chemical. Each application consisted of 10 lambda and any two applications were one day apart. Plants not given cold were treated November 1-5 and plants given cold storage were treated November 15-19. There were three plants per treatment in a randomized block design. Data were taken January 26, 1959, 10 weeks after the final treatment and were analyzed as in experiment 3.

Experiment 5. Plants of the variety Triumph, similar to those used in experiment 3, were sprayed with 10,000 ppm of IAA, TIBA, NAA and GAk in solutions of glucose, boric acid and 95% ethanol. All solutions contained 0.1% Tween 20, and the check was 500 ppm glucose and 500 ppm boric

acid. Plants were sprayed on November 1, 1958. There were four plants per treatment and plants were arranged in a randomized block design. Data were taken January 10, 1959, ten weeks after spraying. Data were collected and analyzed as in experiment 3.

Experiment 6. During the fall of 1959, further spraying experiments were undertaken. Cuttings of the variety Triumph, rooted during the fall of 1958 and topped May 5, 1959, were the plants used in this experiment. The treatments began October 28, 1959. Plants received 0, 2, 4 and 6 weeks of cold storage and sprays of 0, 24.3, 81, 270 and 900 ppm of GAk. Cold treatments were given in an unlighted 37° chamber. GAk was dissolved in a 500 ppm glucose and 500 ppm boric acid solution. Gibberellin solutions were made by dilution of the highest concentration with the glucose-boric acid solution. Each solution contained 0.1% Tween 20. Gibberellin applications were made before or after cold storage treatments. Final treatments of this experiment were applied to plants December 18, 1959. Gibberellin applications were made three times, each application being three days apart. Plants were arranged in a randomized block design, with six plants per treatment. 300 ml. of solution was applied to the six plants of each treatment for the first spraying. The second and third sprayings consisted of 150 ml. of solution applied to the six plants. Plants were grown before and after storage

at a night temperature of 65° F. Data on the percentage that open flowers were of total flower buds were taken four and six weeks after the final treatment was applied. Data was treated in the same manner as the data of experiment 3.

Experiment 7. The second experiment with growth regulators in 1959 involved TIBA, gibberellic acid (GA) and cold temperature. Plants of the variety Triumph were used. These plants were propagated as cuttings during the summer of 1958 and were topped March 5, 1959 and June 12, 1959. TIBA treatments included 0 and 200 ppm, GA treatments included 0 and 1,000 ppm, and cold treatments included no cold storage and 2 weeks of cold storage at 37° F. from November 6 to December 10. This experiment was a 2^3 factorial with six plants per treatment arranged in a completely randomized design. TIBA applications were made December 12 and December 14, and the gibberellic acid application was made on December 16. TIBA was dissolved in 100 ml. 95% ethanol per litre of glucose-boric acid solution. Gibberellic acid was dissolved in 250 ml. 95% ethanol per litre of glucose-boric acid solution. Similar amounts of alcohol appeared in the glucose-boric acid solutions applied to the checks. All solutions contained 0.1% Tween 20. 1200 ml. of each solution was sprayed upon 24 plants. The plants were grown in a greenhouse held at 65° F. at night during the experiment. Data were taken four weeks after the final treatment on the percentage that open flowers were of total

flower buds per plant. Data were handled in the same manner as were the data of experiment 3.

Experiment 8. This experiment was concerned with the effect of NAA upon the rate of flower bud development. Plants of the variety Triumph similar to those mentioned in experiment 6 were used. The plants were sprayed with 0, 2.7, 9, 30 and 100 ppm NAA. The high concentration of NAA contained 250 ml. 95% ethanol per litre as did the check. The glucose-boric acid solution was used, and all solutions contained 0.1% Tween 20. Other concentrations of NAA were made by the dilution method. Sprays were made on November 29, December 2 and December 5, 1959, and amounts of spray applied were the same as those in experiment 6. Treatments were arranged in a completely randomized design with six plants per treatment. Data were taken six weeks after the final treatment on percentage that open flowers were of total flower buds, and were analyzed in the same manner as the data of experiment 3.

Experiment 9. This experiment was undertaken in order to find out the effect of varying concentrations of TIBA in increasing the number of buds per plant. The plants used were similar to those employed in experiment 7. Concentrations of TIBA were 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1,000 ppm in glucose-boric acid solution. All concentrations contained 0.1% Tween 20. Plants were arranged in a completely randomized design with five plants per

treatment. Each treatment received 160 ml. of spray November 29, December 2 and December 5, 1959. Total buds on each plant were counted March 22, 1960. Differences between means were tested by Duncan's multiple range test.

B. EXTRACTION EXPERIMENTS

Experiment 10. Azaleas of the variety Triumph were used for the extraction of endogenous growth substances. These plants were received on June 10, 1958, and grown in the greenhouses at a 65° minimum night temperature. The plants were topped on March 5 and June 23, 1959. In the fall of 1959 extractions were made from plants that had received no cold, 1 week, 2 weeks, 3 weeks, 4 weeks and 6 weeks of cold. Other plants received three sprays of 1,000 ppm GAk, three days apart, and still other plants received four weeks cold plus three sprays of 1,000 ppm GAk. There were 15 plants per treatment, and plants for the cold and GAk treatments and the checks were arranged on the bench of a greenhouse held at 65° F. night temperature in a completely randomized design.

Plants receiving cold storage were placed in the refrigerator October 22, 1959 and were held at 37° F. in the dark. At each of the indicated weekly periods 15 plants were removed from storage and 15 plants from the greenhouse and the swollen flower buds of these plants were extracted for endogenous growth substances. Plants which had received four weeks cold, and plants which had received no cold were

sprayed with GAk on November 21, November 25 and November 29, and were sampled with plants which had received six weeks cold and checks from the greenhouse on December 4. The 30 plants received 1,500 ml. of spray with each spraying. Glucose, boric acid and Tween 20 were used as in experiment 6. At first it was intended to sample plants receiving five weeks of cold too, but some plants were frozen due to a failure in the refrigerator temperature controls. After this mishap all buds had to be examined for dead areas.

The buds removed from the plants were placed in paper bags resting on or between blocks of dry ice in an effort to hold enzymatic activity to a minimum. The following day, after being held at 0° F. overnight, the buds were dried for 36 hours in the Virtis Cryovaporator at a pressure of about 25 microns. The dried buds were ground in a Wiley mill, first with a 20 mesh screen and later with a 40 mesh screen, weighed, and stored in a desiccator at 0° F. in the dark until used.

The extraction method used was essentially that of Nitsch (66) and of Nitsch and Nitsch (68). Aliquots of the equivalent of 20 buds, were transferred to 30 ml of absolute methanol and stored for at least three hours at 0° F. Later washings using 40 ml and finally 50 ml of absolute methanol were carried out. The methanol was removed by means of a rotary evaporator at a temperature of 80° C. and 10 ml acetonitrile was added to the dried extract. The acetonitrile extract was evaporated to 0.25-.50 ml volume, and

this was placed in a stripe upon a 1" wide strip of Whatman 3MM paper with a hypodermic syringe fitted with a blunted 25-guage needle. A vacuum was used to facilitate evaporation of the acetonitrile from the paper.

All chromatograms were run in a solvent consisting of isopropanol (80%), 28% ammonia (10%) and water (10%). This is the IAW solvent of Stowe and Thimann (85). The chromatograms were run in a battery jar fitted with a number 14 rubber stopper through which five glass rods were inserted which moved up and down through glass sleeves. The chromatograms were equilibrated over the solvent for six hours and then the lower two cm. of the chromatogram were inserted into the solvent (the origin being four cm. from the end of the paper.) The solvent was allowed to run 20 cm. from the origin. This took about 10 hours. The chromatograms were then removed from the battery jar and allowed to dry in the open air.

Wheat seedlings of the variety Atlas 66 were soaked for two hours in tap water and placed in a tray. The seeds were spread over several layers of wet facial tissue and the tray was covered with a sheet of clear polyethylene and placed in a germinator. The seeds were allowed to germinate for about 72 hours at 25° C. Coleoptiles that were 25-40 mm. in length were utilized. The first three mm. of the coleoptile were discarded and the next four mm. were used for the bioassay. The sections were cut with Thimann's modification of the Van der Weij coleoptile microtome. The

coleoptile sections were placed in glass-distilled water and kept there for three hours prior to use. These operations were carried out under weak green light of approximately 525 mu.

The chromatograms were cut into two cm. transverse strips, that is, into ten equal sections, and placed in small beakers to which two ml. of 2% sucrose solution were added. The solution contained 0.1% Tween 20 and was buffered at pH5 as recommended by Nitsch and Nitsch (68). The beakers were arranged in a completely randomized design in a glass casserole which contained a small amount of water. Six coleoptile sections were added to each beaker under the green light. This was an increase over the number of coleoptile sections recommended by Walker, Hendershott and Snedecor (92) for such a bioassay with four replications. A glass plate was placed over the casserole and it was placed in the germinator, at 25° C., for 20 hours. The coleoptiles were then placed first upon paper towels to allow them to dry and next upon a glass plate, and were placed in a photographic enlarger, where they received a magnification of 3.88. The images of the coleoptile sections were traced and later measured with a plastic ruler. The mean of the coleoptile lengths for each chromatogram region was determined, and four was subtracted from it, the result being the mean coleoptile growth for a particular chromatogram region of a particular chromatogram. As four chromatograms were produced from each plant treatment, a specific

chromatogram region was considered a treatment with four replicates.

This experiment was divided into five sections. A separate analysis of variance and Duncan's multiple range test of differences between treatment means was run for each section. The first section consisted of a bioassay being run on four chromatograms of the buds from plants receiving one week of cold storage, four chromatograms of buds from plants receiving one week of cold storage and two chromatograms of 100 mg IAA dissolved in acetonitrile. As the IAA covered the first four 2 cm. sections of the chromatogram, the other six sections were used as a check. Similar methods were used on the extracts of plants whose buds had received two, three and four weeks of cold storage. The casserole contained beakers with the sections of two chromatograms of extract from plants which had received cold, two chromatograms of extract of buds which had received no cold and one chromatogram of 100 micrograms IAA. These beakers were arranged in a completely randomized design. Each section, then, was a randomized block experiment.

The final section of experiment 10 consisted of a bioassay run on four chromatograms of buds from plants receiving six weeks of cold storage, GAk sprays, four weeks cold storage and GAk sprays, and no cold storage or GAk sprays. Check chromatogram sections consisted of the bottom two cm. of each chromatogram which was exposed to the solvent but not to the plant extract. Four chromatograms,

one from each plant treatment, were cut up into regions and arranged in the casserole in a completely randomized design. This was done four times. Thus, this bioassay was a randomized block experiment with four replications.

Experiment 11. Single chromatograms of 50 bud extracts of plants receiving no cold or GAk sprays, and of those which had received GAk but no cold were examined with an ultraviolet light, and were sprayed with Ehrlich's reagent (one per cent para-dimethylamino-benzaldehyde in 1N HCl in 80 per cent ethanol.) A chromatogram of 100 micrograms IAA was treated in a similar manner. All chromatography was done as outlined in experiment 10. Other chromatograms of 50 bud extracts of plants receiving no cold or GAk, and of plants receiving GAk but no cold were soaked in 4N H_2SO_4. Chromatograms loaded with 100 micrograms GA and 100 micrograms GAk, both in solutions 75% acetonitrile and 25% absolute ethanol, were also soaked in 4N H_2SO_4 were examined with the ultraviolet light for fluorescence.

Experiment 12. On January 15, 1960, 100 unswollen buds considered to be vegetative and 100 flower buds were removed from plants of the variety Triumph. These plants had received no cold or gibberellin and had received their final topping June 23, 1959. The plants had been grown in the greenhouse at a 65° F. minimum night temperature. As in experiment 10, checks were the bottom two cm. of each chromatogram. Chromatography was carried out as in experiment 10.

The casserole contained beakers with sections of two chromatograms of vegetative bud extract and two chromatograms of flower bud extract arranged in a completely randomized design. This was done twice. Thus, this experiment was a randomized block experiment with two blocks and two replications per block. An analysis of variance was carried out and Duncan's multiple range test was applied to the data.

RESULTS

A. SPRAYING EXPERIMENTS

Experiment 1. The analyses of variance for the four different varieties revealed no significance save for the variety Hexe. Apparently IAA and TIBA had little effect upon the multiple flower bud formation of the four varieties with the exception of variety Hexe. TIBA had an inhibiting effect upon the multiple flower bud formation in Hexe. However, time of application was rather critical, and TIBA sprays were ineffective if made on Hexe July 11. The data indicates that floral initiation may have been taking place about June 20 in the variety Hexe. Data on Hexe appear in Table 1.

Plants that had received six weeks of 40° F. temperature flowered from December 12, 1958 to February 23, 1959, a period exceeding 10 weeks. Plants that were brought in from the cold frame, where they had been stored for six months, flowered over a period of 10 days. Thus, the six weeks cold storage did not result in flowering in a minimum time.

Plants sprayed with the two higher concentrations of IAA displayed hyponasty of the upper leaves and stem. The high concentration of TIBA caused a slight epinasty of the upper leaves and an upward curling along the edges of the leaves.

TABLE 1

PER CENT ONE- AND TWO-FLOWERED BUDS OF HEXE AZALEAS SPRAYED WITH IAA AND TIBA

Treatment	Per cent one- and two flowered buds
June 20 sprays:	
Check	62.8[1]
50 ppm IAA	65.1
150 ppm IAA	72.7
450 ppm IAA	71.5
50 ppm TIBA	75.6*[2]
150 ppm TIBA	84.4*
450 ppm TIBA	75.4*
July 11 sprays:	
Check	67.7
50 ppm IAA	68.5
150 ppm IAA	67.7
450 ppm IAA	66.3
50 ppm TIBA	74.6
150 ppm TIBA	72.4
450 ppm TIBA	71.1
June 20 and July 11 sprays:	
Check	70.5
50 ppm IAA	72.9
150 ppm IAA	62.5
450 ppm IAA	70.7
50 ppm TIBA	79.2
150 ppm TIBA	82.0*
450 ppm TIBA	81.0*

[1]Check means are means of 16 replications; other means are means of eight replications--all means calculated using arc sin transformations.

[2]Means followed by an asterisk are significantly different from the check at the 5% level.

Experiment 2. Data of experiment 2 appear in Table 2. As all of these applications were made on a single date (July 3) when the time of flower bud formation may well have passed, the only conclusion from this experiment is that IAA may stimulate multiple flower bud formation.

Experiment 3. The data of experiment 3 appear in Table 3. All of the checks that had received five weeks of cold died. All plants that received 1,000 ppm NAA died. The buds of all plants that received 400 ppm NAA were killed as were those that received 1,000 ppm TIBA with three and five weeks of cold, and those that received 3 weeks cold and 1,000 ppm GAk. Table 3 indicates that there were no significant differences between treatments for plants that had received no cold, and plants that had received one week of 45° F. storage. Data for plants that had received no sprays indicates that the varying cold periods alone did not affect the plants greatly.

After two weeks cold some of the TIBA treatments provided a significant increase in the rate of flower bud development over the check, as did the 400 and 1,000 ppm IAA treatments and the 400 ppm GAk treatment after three weeks of cold. After four weeks of cold there were few significant differences between chemical treatments. However, the 1,000 ppm GAk treatment did give the greatest stimulus to flower bud development. After five weeks of cold the greatest increase in flower bud development was achieved by plants

TABLE 2

PER CENT ONE-FLOWERED BUDS OF VERVAENEANA AZALEAS SPRAYED WITH IAA AND TIBA

Treatment	Per cent one-flowered buds
Check	50.5[1]
200 ppm IAA	34.0
400 ppm IAA	46.0
600 ppm IAA	55.9
800 ppm IAA	31.6
1000 ppm IAA	56.0
1200 ppm IAA	52.6
1400 ppm IAA	39.3
1600 ppm IAA	66.6
1800 ppm IAA	54.8
2000 ppm IAA	44.8
2200 ppm IAA	23.6*[2]
2400 ppm IAA	54.6
200 ppm TIBA	46.4
400 ppm TIBA	28.9
600 ppm TIBA	53.2
800 ppm TIBA	40.8
1000 ppm TIBA	66.5
1200 ppm TIBA	62.5
1400 ppm TIBA	64.3
1600 ppm TIBA	37.2
1800 ppm TIBA	32.8
2000 ppm TIBA	67.7
2200 ppm TIBA	66.8
2400 ppm TIBA	35.3

[1]Check mean is a mean of six replications; other means are means of three replications--all means calculated using arc sin transformations.

[2]Mean followed by an asterisk is significantly different from the check at the 5% level.

TABLE 3

INFLUENCE OF COLD STORAGE TREATMENTS AND GROWTH REGULATOR SPRAYS UPON FLOWER BUD DEVELOPMENT OF TRIUMPH AZALEAS EIGHT WEEKS AFTER FINAL TREATMENTS

Chemical	Conc. (ppm)	Per cent open flowers of total flower buds					
		0 weeks cold	1 week cold	2 weeks cold	3 weeks cold	4 weeks cold	5 weeks cold
-	-	2.6[1] a-h[2]	0.8 abc	8.3 a-p	7.9 a-p	19.9 a-w	-
IAA	64	3.1 a-i	6.5 a-n	3.7 a-i	21.0 a-w	39.6 i-x	39.2 i-x
	160	0 a	12.2 a-t	10.3 a-r	5.7 a-l	42.8 k-x	36.2 f-x
	400	0 a	6.1 a-m	7.4 a-p	54.6 r-y	16.8 a-u	56.7 t-y
	1000	0 a	1.3 a-d	19.0 a-v	55.8 s-y	34.9 e-x	12.2 a-t
TIBA	64	0.3 ab	3.8 a-i	14.2 a-t	29.6 c-x	32.7 d-x	55.2 r-y
	160	1.2 a-d	10.7 a-s	43.5 k-x	37.3 g-x	4.4 a-k	53.5 g-y
	400	2.0 a-f	12.9 a-t	43.8 l-x	47.0 m-y	17.3 a-v	40.5 i-x
	1000	0 a	2.1 a-g	48.5 p-y	-	30.7 c-x	-
NAA	64	1.5 a-e	0 a	33.1 d-x	34.1 e-x	44.1 l-x	14.6 a-u
	160	1.4 a-d	1.1 a-d	2.3 a-g	38.5 h-x	40.2 i-x	3.3 a-i
GAk	64	0 a	3.9 a-j	18.4 a-v	27.2 b-x	42.5 j-x	44.9 l-x
	160	9.6 a-q	3.4 a-i	8.8 a-p	29.1 c-x	21.8 a-x	59.6 u-y
	400	3.8 a-i	5.6 a-l	3.8 a-i	65.1 v-y	19.3 a-w	68.0 wxy
	1000	1.5 a-e	12.9 a-t	25.5 b-x	-	47.9 n-y	89.0 y

[1]Mean of four replications calculated with arc sin transformations.

[2]Means followed by the same letters are not significantly different at the 5% level.

sprayed with 400 and 1000 ppm GAk.

Apparently plants in this experiment had been treated with insufficient quantities of the chemicals for clearcut results. Higher concentrations of GAk did provide some stimulation in combination with the varying cold periods, but it was not clear whether this effect was upon termination of bud dormancy or upon shortening the forcing period. Higher concentrations of IAA and TIBA also apparently had some effect upon flower bud development but results with these chemicals were not as consistent as with GAk. On the basis of these results, the following year the effects of GAk and cold storage upon the rate of flower bud development were studied further.

Experiment 4. The analysis of variance of this experiment indicated no significant differences between treatments. Apparently, either insufficient chemical reached the buds, or the chemical, to be effective must be applied to the leaves. Buds which had been treated with 30 lambda or more of NAA were dead.

Experiment 5. No significant differences between treatments were indicated by the analysis of variance. Plants treated with 10,000 ppm NAA died as did two of the four plants treated with 10,000 ppm TIBA. A large number of vegetative shoots appeared on the remaining two plants a month after spraying, as if there had been a complete removal of apical dominance. A similar increase in vegetative shoot

production was noted in a few of the plants in experiment 3 that had received two weeks cold and a spray of 1,000 ppm TIBA (Fig. 1). Apical dominance did not reappear in these plants until a year after spraying. 10,000 ppm applications of other chemicals apparently had little effect upon flower bud dormancy.

Experiment 6. Data from experiment 6 was collected on January 14, 1960, four weeks after final treatments, and January 28, 1960, six weeks after final treatments. Data of experiment 6 taken four weeks after final treatments appear in Table 4. Apparently, for January 14 flowering, date of removal from cold storage as well as amount of cold storage were important. Four weeks cold ending November 25 provided earlier flowering than no cold, four weeks cold ending December 4 or six weeks cold. Plants that had received no cold or two weeks of cold and sprayed with 900 ppm GAk were significantly different from plants that had received no cold and no GAk. However, only in the case of plants receiving no cold at all could applications of 900 ppm GAk provide a significant increase in flowering over plants that received no chemical. Apparently GAk is effective whether applied before or after the cold storage period. Equations were fitted to the data for plants receiving no GAk and 900 ppm GAk in order to show trends due to cold storage (Fig. 2). The equation $y = 31.61 + 5.41x - 1.77x^2$ was fitted for plants receiving 900 ppm GAk after cold storage. The equation for

Fig. 1. Effect of TIBA upon apical dominance.
At left--2 weeks cold. At right--2 weeks cold and 1000 ppm TIBA.

TABLE 4

FLOWER BUD DEVELOPMENT OF TRIUMPH AZALEAS FOUR WEEKS AFTER COLD STORAGE AND GAk SPRAY TREATMENTS

Treatment			Per cent open flowers of total flower buds	
Weeks of cold	Time of application	Concentration (ppm)		
0		0	3.0[1]	a-d[2]
		24.3	8.0	a-f
		81.0	9.1	a-g
		270.0	16.0	c-g
		900.0	27.7	efg
2 (10/28-11/11)	After Storage	0	12.7	b-g
		24.3	15.6	c-g
		81.0	11.4	a-g
		270.0	9.9	a-g
		900.0	32.7	fg
2 (11/6-11/20	Before Storage	0	13.6	b-g
		24.3	8.7	a-g
		81.0	18.1	d-g
		270.0	20.5	d-g
		900.0	36.0	g
4 (10/28-11/25)	After Storage	0	33.9	fg
		24.3	16.5	d-g
		81.0	27.9	fg
		270.0	14.8	1-g
		900.0	18.3	d-g
4 (11/6-12/4)	Before Storage	0	4.1	a-d
		24.3	0	a
		81.0	0	a
		270.0	2.7	a-d
		900.0	4.3	a-e
6 (10/28-12/10)	After Storage	0	0	a
		24.3	0.4	ab
		81.0	0.6	ab
		270.0	0	a
		900.0	0	a
6 (11/6-12/18)	Before Storage	0	0	a
		24.3	0	a
		81.0	0	a
		270.0	0	a
		900.0	0	a

[1]Mean of six replications calculated using arc sin transformations.

[2]Means followed by the same letter or letters are not significantly different at the 5% level.

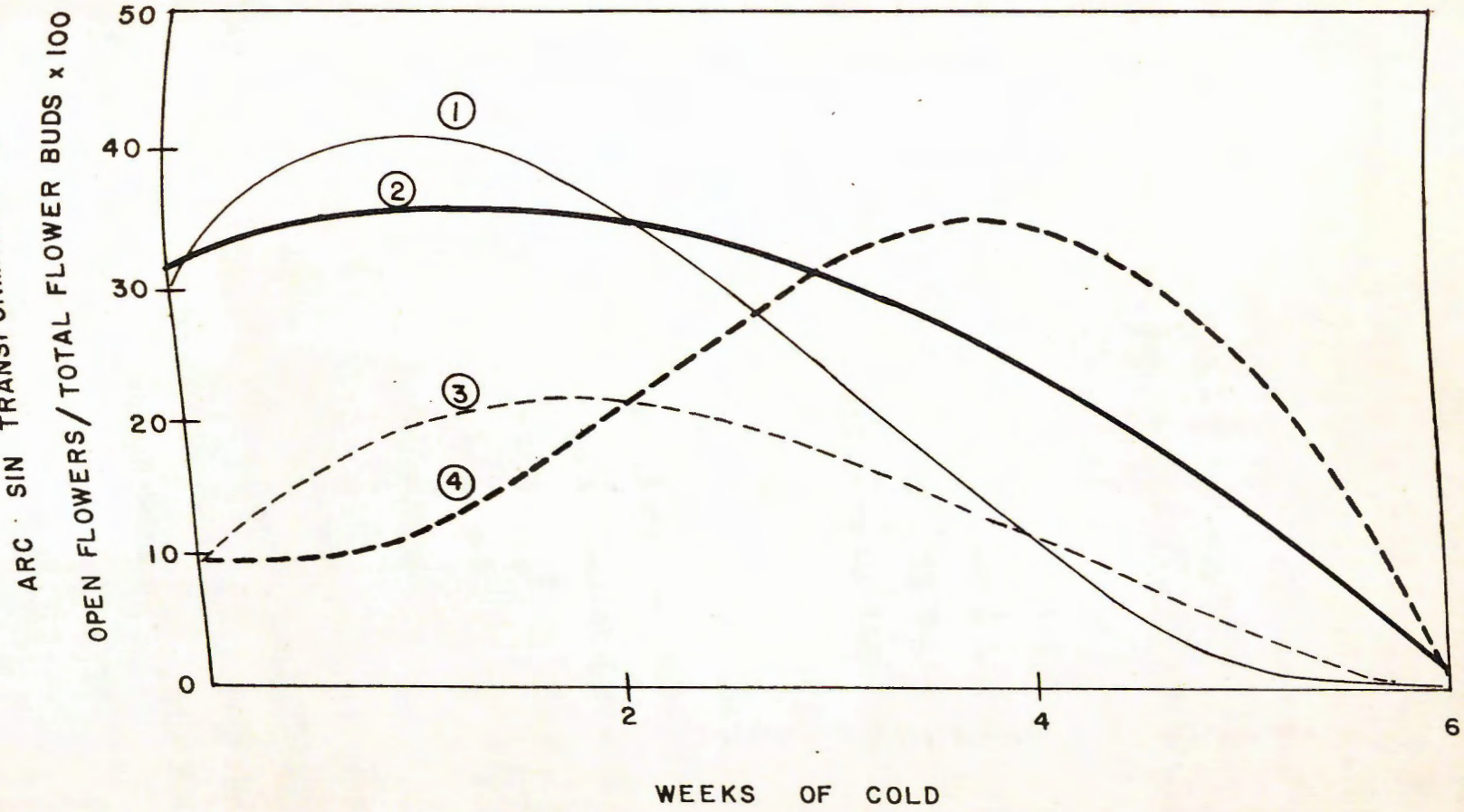

Fig. 2. Influence of cold storage and GAk sprays upon flower bud development of Triumph azaleas four weeks after final treatment.
1. 900 ppm GAk applied before cold storage.
2. 900 ppm GAk applied after cold storage.
3. 0 ppm GAk applied before cold storage.
4. 0 ppm GAk applied after cold storage.

the corresponding check was $y = 9.88 - 4.52x + 7.23x^2 - 1.13x^3$. The equation for plants receiving 900 ppm GAk before cold storage was $y = 31.81 + 17.14x - 9.09x^2 + 0.89x^3$ and the equation for the corresponding check was $y = 9.92 + 14.53x - 5.18x^2 + 0.41x^3$.

Data of experiment 6 taken six weeks after final treatment appear in Table 5. According to Table 5, six weeks of cold results in slower flowering than four weeks of cold or no cold. GAk had little effect upon rate of flower bud development if data were taken six weeks after final treatment, except for the plants receiving no cold. However, the 900 ppm treatment was not significantly greater than the check, but was significantly greater than the 24.3 ppm treatment.

Experiment 7. The treatment means of experiment 7 appear in Table 6. The data indicate that a combination of TIBA and GAk is effective in increasing the rate of flower bud development, as is a combination of TIBA and cold. The results with TIBA and two weeks cold storage are consistent with results reported for experiment 3.

Experiment 8. The treatment means of experiment 8 appear in Table 7. As the concentration of NAA increases the rate of flower bud development decreases. Many of the buds sprayed with 100 ppm NAA were dead. The equation $y = 39.223 - 20.217 \log x$ was fitted to the data to describe the effect of NAA (Fig. 3).

TABLE 5

FLOWER BUD DEVELOPMENT OF TRIUMPH AZALEAS SIX WEEKS AFTER COLD STORAGE AND GAk SPRAY TREATMENTS

Treatment			Per cent open flowers of total flower buds	
Weeks of cold	Time of application	Concentration (ppm)		
0		0	53.8[1]	d-g[2]
		24.3	22.3	abc
		81.0	40.8	b-g
		270.0	35.4	a-f
		900.0	65.5	efg
2 (10/28-11/11)	After Storage	0	38.9	b-g
		24.3	42.0	b-g
		81.0	49.6	d-g
		270.0	33.0	a-f
		900.0	65.1	efg
2 (11/6-11/20)	Before Storage	0	33.5	a-f
		24.3	52.5	d-g
		81.0	42.3	b-g
		270.0	41.6	b-g
		900.0	48.7	d-g
4 (10/28-11/25)	After Storage	0	69.0	fg
		24.3	67.0	fg
		81.0	56.5	d-g
		270.0	53.9	d-g
		900.0	76.4	g
4 (11/6-12/4)	Before Storage	0	53.9	d-g
		24.3	56.1	d-g
		81.0	33.4	a-f
		270.0	44.4	b-f
		900.0	46.2	c-g
6 (10/28-12/10)	After Storage	0	31.2	a-f
		24.3	31.2	a-f
		81.0	25.7	a-e
		270.0	40.5	b-g
		900.0	41.3	b-g
6 (11/6-12/18)	Before Storage	0	5.1	a
		24.3	32.8	a-f
		81.0	10.5	abc
		270.0	9.4	ab
		900.0	31.9	a-f

[1]Mean of six replications calculated using arc sin transformations.

[2]Means followed by the same letter or letters are not significantly different at the 5% level.

TABLE 6

INFLUENCE OF TIBA, GA AND COLD STORAGE UPON FLOWER BUD DEVELOPMENT OF TRIUMPH AZALEAS SIX WEEKS AFTER FINAL TREATMENTS

Treatment			Per cent open flowers
Weeks of cold	TIBA (ppm)	GA (ppm)	
0	0	0	11.1[1] b[2]
0	200	0	8.4 b
0	0	1,000	11.6 b
0	200	1,000	35.8 c
2	0	0	0 a
2	200	0	14.1 b c
2	0	1,000	0 a
2	200	1,000	3.4 a b

[1]Mean of six replications calculated using arc sin transformations.

[2]Means followed by the same letter are not significantly different at the 5% level.

TABLE 7

INFLUENCE OF NAA UPON FLOWER BUD DEVELOPMENT OF TRIUMPH AZALEAS SIX WEEKS AFTER FINAL TREATMENT

Concentration of NAA (ppm)	Per cent open flowers	
0	52.3[1]	c[2]
2.7	29.9	c
9.0	7.5	b
30.0	2.8	a b
100.0	0	a

[1]Mean of six replications calculated using arc sin transformations.

[2]Means followed by the same letters are not significantly different at the 5% level.

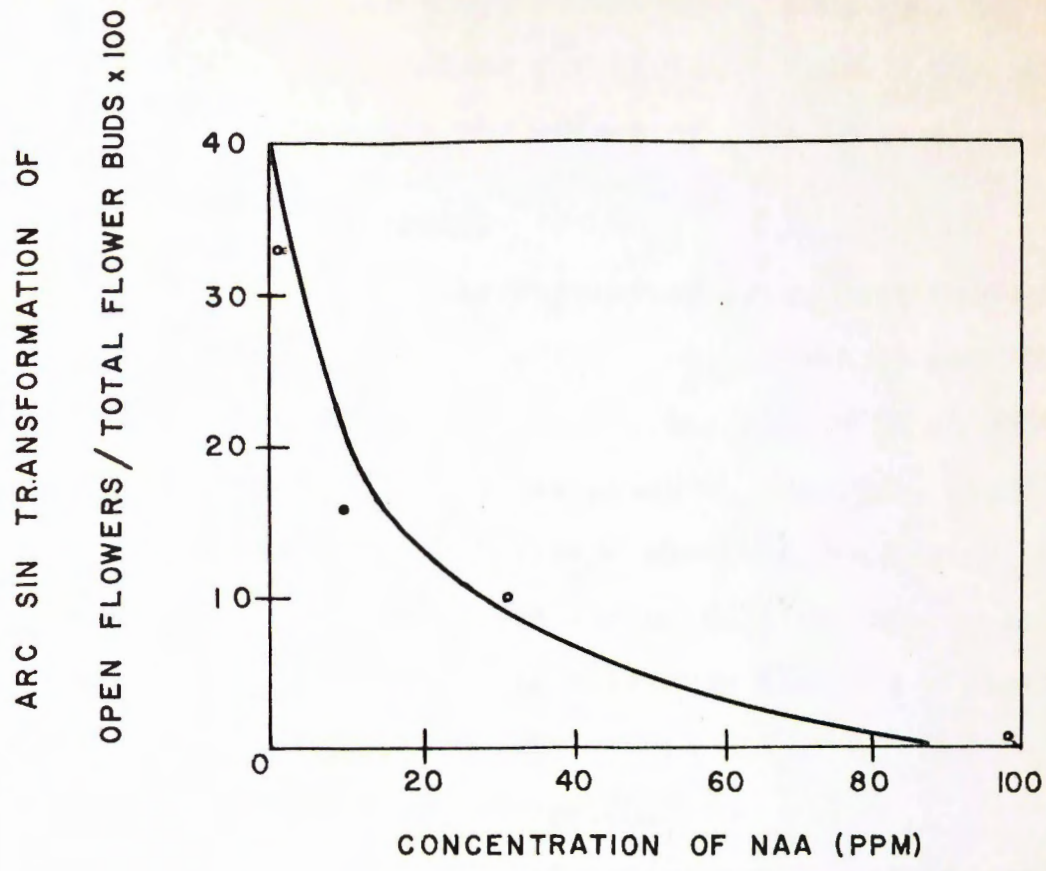

Fig. 3. Inhibition of flower bud development by NAA.

Experiment 9. The treatment means of experiment 9 appear in Table 8. Table 8 indicates that a significant increase in total buds was achieved by plants receiving 800 and 900 ppm TIBA over those receiving 0, 100, 200, 300 and 400 ppm TIBA. The equation $y = 17.136 + 0.026 x$ was fitted to the data to describe the effect of TIBA (Fig. 4).

B. EXTRACTION EXPERIMENTS

Experiment 10. The results of experiment 10 appear in Tables 9 to 13. From these tables, it can be seen that after three weeks of 37° F storage the region of coleoptile growth inhibition is no longer present. However, significant differences between the same chromatogram region of plants receiving cold storage and no cold storage do not appear until after four weeks cold when there is a significant difference between treatments in the Rf 0.5-0.6 region. This is a growth inhibiting region.

In the final section of experiment 10, plants that had received no cold storage or GAk and no cold storage had a region of growth inhibition significantly different from the check. Again, the only difference between chromatogram regions of the four treatments was in the Rf 0.5-0.6 region between plants receiving six weeks cold storage and no cold storage.

Experiment 11. The chromatogram of buds sprayed with GAk and that not sprayed as well as the chromatogram of 100 micrograms IAA displayed a blue fluorescence in the Rf

TABLE 8

EFFECT OF TIBA UPON VISIBLE BUDS OF TRIUMPH AZALEAS FIFTEEN WEEKS AFTER THE FINAL SPRAY

Concentration of TIBA (ppm)	Visible Buds	
0	19.8[1]	a[2]
100	23.0	a
200	23.0	a
300	26.4	a
400	21.6	a
500	34.0	a b
600	35.0	a b
700	30.6	a b
800	46.0	b
900	48.2	b
1000	38.2	a b

[1]Means of five replications.

[2]Means followed by the same letters are not significantly different at the 5% level.

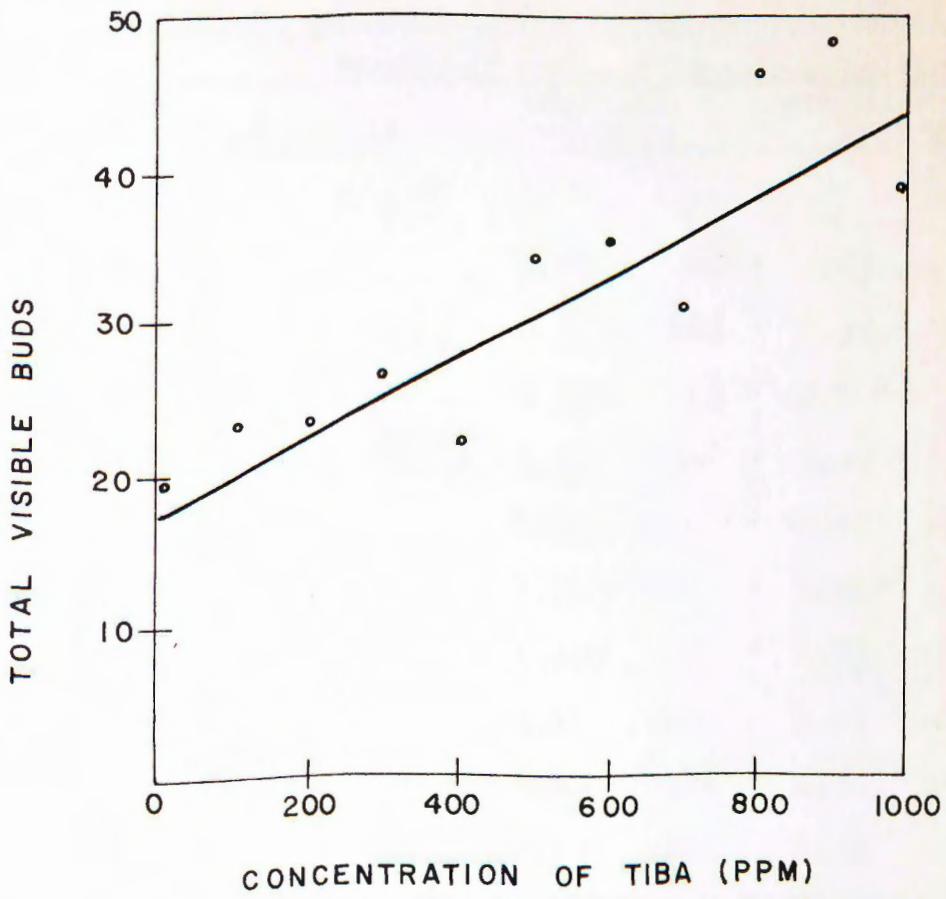

Fig. 4. Removal of apical dominance by TIBA.

TABLE 9

LINEAR GROWTH OF WHEAT COLEOPTILE SECTIONS EXPOSED TO CHROMATOGRAM REGIONS OF TRIUMPH AZALEA FLOWER BUD EXTRACTS FROM PLANTS EXPOSED TO ONE WEEK OF COLD STORAGE AND NO COLD STORAGE

Chromatogram region (Rf)	Growth of coleoptile sections (mm)					
	Check	IAA	no cold storage		one week cold storage	
	2.16[1]	3.11*[2]				
0.0 - 0.1			2.50	cd[3]	2.45	cd
0.1 - 0.2			2.32	bcd	2.32	bcd
0.2 - 0.3			2.23	bcd	2.76*	d
0.3 - 0.4			1.91	abc	2.09	abcd
0.4 - 0.5			1.95	abc	1.81	abc
0.5 - 0.6			1.71	ab	1.41*	a
0.6 - 0.7			1.42*	a	1.93	abc
0.7 - 0.8			2.17	bcd	2.13	abcd
0.8 - 0.9			2.42	bcd	1.98	abc
0.9 - 1.0			1.80	abc	2.03	abc

[1]Check mean is mean of 12 replications; IAA mean is mean of eight replications; other means are means of four replicates.

[2]Means followed by an asterisk are significantly different from the check at the 5% level.

[3]Means followed by the same letters are not significantly different at the 5% level.

TABLE 10

LINEAR GROWTH OF WHEAT COLEOPTILE SECTIONS EXPOSED TO CHROMATOGRAM REGIONS OF TRIUMPH AZALEA FLOWER BUD EXTRACTS FROM PLANTS EXPOSED TO TWO WEEKS OF COLD STORAGE AND NO COLD STORAGE

Chromatogram region (Rf)	Growth of coleoptile sections (mm)					
	Check	IAA	no cold storage		two weeks cold storage	
	1.99[1]	2.94*[2]				
0.0 - 0.1			1.82	b-e[3]	1.88	cde
0.1 - 0.2			2.04	de	2.12	e
0.2 - 0.3			1.85	b-e	2.03	de
0.3 - 0.4			1.63	a-e	1.82	b-e
0.4 - 0.5			1.62	a-e	1.75	a-e
0.5 - 0.6			1.27*	ab	1.45*	a-d
0.6 - 0.7			1.33*	abc	1.17*	a
0.7 - 0.8			1.99	de	1.73	a-e
0.8 - 0.9			2.11	e	1.88	cde
0.9 - 1.0			1.80	b-e	1.98	de

[1]Check mean is mean of 12 replications; IAA mean is mean of eight replications; other means are means of four replicates.

[2]Means followed by an asterisk are significantly different from the check at the 5% level.

[3]Means followed by the same letters are not significantly different at the 5% level.

TABLE 11

LINEAR GROWTH OF WHEAT COLEOPTILE SECTIONS EXPOSED TO CHROMATOGRAM REGIONS OF TRIUMPH AZALEA FLOWER BUD EXTRACTS FROM PLANTS EXPOSED TO THREE WEEKS OF COLD STORAGE AND NO COLD STORAGE

Chromatogram region (Rf)	Check	IAA	no cold storage		three weeks cold storage	
	1.66[1]	2.43*[2]				
0.0 - 0.1			1.72	bcd[3]	1.39	abc
0.1 - 0.2			1.73	bcd	1.49	abcd
0.2 - 0.3			1.72	bcd	2.05	d
0.3 - 0.4			1.62	abcd	1.59	abcd
0.4 - 0.5			1.55	abcd	1.39	abc
0.5 - 0.6			1.15*	a	1.43	abc
0.6 - 0.7			1.27	ab	1.34	abc
0.7 - 0.8			1.66	abcd	1.37	abc
0.8 - 0.9			1.73	bcd	1.95	cd
0.9 - 1.0			1.60	abcd	1.79	bcd

[1]Check mean is mean of 12 replications; IAA mean is mean of eight replications; other means are means of four replications.

[2]Means followed by an asterisk are significantly different from the check at the 5% level of significance.

[3]Means followed by the same letters are not significantly different at the 5% level.

TABLE 12

LINEAR GROWTH OF WHEAT COLEOPTILE SECTIONS EXPOSED TO CHROMATOGRAM REGIONS OF TRIUMPH AZALEA FLOWER BUD EXTRACTS FROM PLANTS EXPOSED TO FOUR WEEKS OF COLD STORAGE AND NO COLD STORAGE

Chromatogram region (Rf)	Growth of coleoptile sections (mm)					
	Check	IAA	no cold storage		four weeks cold storage	
	1.61[1]	1.88				
0.0 - 0.1			1.86	d-g[2]	1.91	efg
0.1 - 0.2			1.55	b-g	1.61	c-g
0.2 - 0.3			1.65	d-g	1.67	d-g
0.3 - 0.4			1.35	bcd	1.51	b-f
0.4 - 0.5			1.05*	ab[3]	1.27	bc
0.5 - 0.6			0.65*	a	1.33	bcd
0.6 - 0.7			1.41	b-e	1.73	d-g
0.7 - 0.8			2.07*	g	2.02	fg
0.8 - 0.9			1.84	d-g	2.04	fg
0.9 - 1.0			1.95	efg	1.71	d-g

[1]Check mean is mean of 12 replications; IAA mean is mean of eight replications; other means are means of four replications.

[2]Means followed by the same letters are not significantly different at the 5% level.

[3]Means followed by an asterisk are significantly different from the check at the 5% level of significance.

TABLE 13

LINEAR GROWTH OF WHEAT COLEOPTILE SECTIONS EXPOSED TO CHROMATOGRAM REGIONS OF TRIUMPH AZALEA FLOWER BUD EXTRACTS FROM PLANTS EXPOSED TO SIX WEEKS COLD, FOUR WEEKS COLD AND GAk, NO COLD AND GAk, AND NO COLD OR GAk

Chromatogram region (Rf)	Growth of coleoptile sections (mm)				
	Check	no cold no GAk	six weeks cold no GAk	four weeks cold GAk	no cold GAk
	1.64[1]				
0.0 - 0.1		2.07*[2] fg[3]	1.66 b-g	1.69 c-g	1.87 d-g
0.1 - 0.2		1.74 c-g	1.53 a-e	1.69 c-g	1.71 c-g
0.2 - 0.3		1.73 c-g	1.53 a-e	1.80 d-g	1.77 d-g
0.3 - 0.4		1.75 c-g	1.76 d-g	2.18* g	2.01 efg
0.4 - 0.5		1.42 a-d	1.75 c-g	1.80 d-g	1.53 a-e
0.5 - 0.6		1.15* ab	1.72 c-g	1.52 a-e	1.22*abc
0.6 - 0.7		1.10* a	1.59 a-f	1.44 a-e	1.40 a-d
0.7 - 0.8		1.80 d-g	1.65 b-g	2.01 efg	1.92 d-g
0.8 - 0.9		2.14* fg	1.83 d-g	1.90 d-g	2.08* fg
0.9 - 1.0		1.81 d-g	1.55 a-f	1.77 d-g	1.79 d-g

[1]Check mean is mean of 16 replications; all other means are means of four replications.

[2]Means followed by an asterisk are significantly different from the check at the 5% level.

[3]Means followed by the same letter are not significantly different at the 5% level.

0.3 - 0.5 region, while the IAA spot appeared greyish-blue. Chlorophyll could have masked any fluorescence or Ehrlich-positive spots in the Rf 0.7 - 1.0 region of the plant extract chromatograms.

The chromatogram of 100 micrograms GA, and the chromatogram of 100 micrograms GAk both showed a light blue fluorescence in the Rf 0.9 - 1.0 region when soaked in 4N H_2SO_4 and exposed to ultra-violet light. Both chromatograms of plant extract similarly treated displayed a bright blue fluorescence in the Rf 0.3 - 0.5 region.

Experiment 12. The means of experiment 12 appear in Table 14. The only chromatogram region where there is a significant difference between vegetative and flowering buds is in the Rf 0.5 - 0.6 region of inhibition.

TABLE 14

LINEAR GROWTH OF WHEAT COLEOPTILE SECTIONS EXPOSED TO CHROMATOGRAM REGIONS OF EXTRACTS OF VEGETATIVE AND FLOWER BUDS OF TRIUMPH AZALEAS

Chromatogram region (Rf)	Growth of coleoptile sections (mm)			
	Check	Vegetative buds	Flowering buds	
	1.92[1]			
0.0 - 0.1		2.00 b-f[2]	2.10	c-g
0.1 - 0.2		1.91 b-e	1.79	bc
0.2 - 0.3		1.91 b-e	2.02	c-g
0.3 - 0.4		2.17 c-g	2.00	b-f
0.4 - 0.5		2.14 c-g	1.83	bcd
0.5 - 0.6		1.90 b-e	1.34*[3]	a
0.6 - 0.7		1.87 b-e	1.59	ab
0.7 - 0.8		2.77 efg	2.10	c-g
0.8 - 0.9		2.24 d-g	2.36*	fg
0.9 - 1.0		2.43* g	2.38*	fg

[1]Check mean is mean of eight replications; all other means are means of four replications.

[2]Means followed by the same letter are not significantly different at the 5% level.

[3]Means followed by an asterisk are significantly different from the check at the 5% level.

DISCUSSION

Although the data on multiple flower bud formation is somewhat limited, it does indicate that IAA may promote multiple flower bud formation and that TIBA may reduce it. The data on Hexe in experiment 1 indicates the importance of the time of application of the chemicals. A June 20 spray, and June 20 and July 11 sprays were effective in promoting multiple flower bud formation while the July 11 spray alone was not. Flower bud initiation must have been taking place around June 20 in Hexe. In the other varieties the time of flower bud initiation had probably passed before growth regulators were applied. If sprays had been more frequent, and had not been applied so long after topping, more informative results might have been achieved with these experiments. Also, had IAA been applied in higher concentrations, as in experiment 2, greater multiple flower bud promotion might have been realized. Earlier experiments have demonstrated the effectiveness of nitrogen fertilization in increasing multiple flower bud formation (46, 82). Auxins might also have such an effect.

In experiment 3 the 1,000 ppm GAk was most effective in removing flower bud dormancy after four or five weeks of cold. This is not surprising, considering the effectiveness of GA in removing the low temperature requirement of other

plants (4, 5, 17, 47, 48). The effectiveness of higher concentrations of IAA after three weeks cold could be explained by the fact that if GA is effective by acting on an auxin destruction system (30), then auxin may have a similar effect to GA. The GAk sprays would have been more effective had they been supplied more frequently. Boodley and Mastalerz (11) used up to eight weekly applications of 1,000 ppm GAk. Apparently TIBA was most effective in combination with two weeks of cold storage. If auxin plays a part in the removal of flower bud dormancy then it is possible that TIBA can act as an auxin synergist (87) with the auxin already present in the bud. NAA did not act as expected. It was thought that if the removal of flower bud dormancy was an auxin response, that NAA would have a greater effect than IAA because it would not be attacked by IAA oxidase. However, its only effects were toxic. Possibly the chemical should be applied in lower concentrations.

The micropipette applications of experiment 4 were ineffective, possibly because of the small amount of chemical applied. The most each bud received was 50 lambda, whereas each plant received about 125 ml. of solution. It could be possible that the chemicals must be absorbed by the leaves, or must be changed in some manner by the leaves, before becoming effective in the development of the bud.

Of the 10,000 ppm applications the GAk was probably ineffective because it was applied as a single application and all of the chemical could not be absorbed or translocated

at one time. The loss in apical dominance due to 10,000 ppm TIBA treatments, or a few weeks cold and 1,000 ppm TIBA is similar to the effect the chemical has had upon roses as reported by Asen and Hamner (2). It is possible that TIBA reduces the natural auxin supply in some manner, maybe by inhibiting its translocation (34). Thimann and Skoog (88) have shown that auxins produced at the tip of the plant inhibit growth of lateral buds and so control the branching habit. This could be the case in the azalea.

In experiment 6, cold treatment apparently had a greater effect upon flower bud development of Triumph azaleas than had GAk applications (Fig. 2). Four weeks cold was optimum and plants exposed to four weeks cold were not stimulated by GAk. However, no cold and 900 ppm GAk or two weeks cold and 900 ppm GAk provided a significant increase over no cold and 0 ppm GAk, as did four weeks of cold storage. Data taken six weeks after final treatment do not indicate these differences. The time at which data are taken is important in regard to the analysis of results of such an experiment. The effectiveness of GAk in combination with no cold or two weeks cold in experiment 6 compared with its ineffectiveness with no cold or two weeks cold in experiment 3 is an indication of the importance of more applications of the chemical.

Some of the results of cold storage and GAk treatments of experiment 6 may be explained in part by the wheat coleoptile bioassay of experiment 10. It should be stressed

that the inhibitors and promoters referred to here have only inhibited or promoted the growth of sections of wheat coleoptile and have not been applied to azalea flower buds. While the inhibitor of the Rf 0.5 - 0.6 region is no longer present in inhibiting concentrations after three weeks of cold storage, it is still present in plants treated with GAk and no cold. Also, plants which have received six weeks cold do not have promoters present that are present in plants receiving no cold or four weeks cold. These promoters could not be detected statistically in plants receiving no, one, two or three weeks of cold and they may not be an important factor. However, they could be detected in buds removed later from the plants. The promoters could have been respired in storage and were not resynthesized, either because of a lack of photosynthesis or a lack of light for some other process. As the inhibitor was not present in the vegetative buds sampled in experiment 12 the inhibitor is probably synthesized some time during floral initiation or the period following initiation. Possibly, the inhibitor is respired in storage and cannot be resynthesized until removal from storage because of a lack of photosynthesis or some other process affected by light. However, the data indicate that there may be a resynthesis of the inhibitor after plants are put back into the greenhouse (see data for 4 weeks cold and GAk in Table 13).

Pilet and Wurgler (72) and McCune and Galston (63) have found that GA can cause a decrease in auxin oxidase

activity. If this is the case in Triumph azalea buds treated with GAk, then an auxin would appear or increase in concentration. Unless an inhibitor was part of auxin oxidase it would not be affected by GAk, and, in plants sprayed with GAk there is a region of inhibition. In addition, in plants treated with four weeks cold storage and GAk, a growth promoter appeared in the Rf 0.3 - 0.4 region. Phillips, Vlitos and Cutter (69) have reported growth active zones appearing in chromatograms of extracts of peas treated with GA. GAk may be responsible for the appearance of the promoter as in experiment 10. Experiment 11 demonstrates that IAA may be found in the Rf 0.3 - 0.4 region of chromatograms run under the conditions of experiment 10, but chromatograms of plant extract were Ehrlich negative despite the fluorescence they had in the Rf 0.3 - 0.4 region. Possibly some compound that becomes pink with Ehrlich's reagent might have masked any IAA present, but in any case, it cannot be concluded that IAA was present in the plant extract. As both GA and GAk appeared in the Rf 0.9 - 1.0 region, neither of them was the promoter found in the 0.3 - 0.4 region. It is possible that GAk and cold storage affect the buds differently; GAk by influencing the buildup of a promoter and cold by causing the breakdown of an inhibitor. Experiment 7 indicates that GA and cold storage both react with TIBA to remove flower bud dormancy.

The inhibitor cannot be considered to be naringenin that Hendershott and Walker (37) identified in dormant peach

flower buds, because there was a lack of fluorescence in ultra-violet light by the inhibiting Rf 0.5 - 0.6 region.

The delaying effect of NAA upon flower bud development in experiment 8 may be compared with the delay the chemical causes in chrysanthemums. Tsukamoto and Harada (89) found that a spray of 50 ppm NAA or more delayed the flowering of chrysanthemums if applied before differentiation. In experiment 8 the lower concentrations of NAA apparently do not damage the flower buds of the azaleas as did the 100 ppm treatment. The delay effect could have been due to some toxic effect, or possibly, to an auxin concentration above optimum.

SUMMARY AND CONCLUSIONS

Experiments were conducted to compare the effectiveness of various plant growth regulators upon multiple flower bud formation, and upon removal of flower bud dormancy of evergreen azaleas. The chemicals were applied as foliar sprays, and plants were sprayed until runoff occurred. Varieties used were Triumph, Vervaeneana, Hexe, Dorothy Gish and Rose Greeley. An attempt was also made to find the effect of cold storage and GAk sprays upon levels of endogenous growth substances within dormant flower buds and within vegetative buds. Paper chromatograms of methanol extractions were run in isopropanol: ammonia: water (8/1/1) and sections of chromatograms were tested by their influence on growth of wheat coleoptile sections.

All experiments had a completely randomized design or a randomized block design. Duncan's multiple range test was used to test differences between treatment means except in the multiple flower bud experiments where the least significant difference was employed. The 5% level of significance was used in all experiments. Where trends were obvious, equations were fitted to the data by the method of orthogonal polynomials.

The spraying experiments indicated that:

(1) IAA may stimulate multiple flower bud formation

and TIBA may inhibit multiple flower bud formation;

(2) Time of application is exceedingly important if auxin or anti-auxin sprays are to be used to modify multiple flower bud formation;

(3) Removal of flower bud dormancy can be stimulated by:

a) Two weeks cold and single sprays of 160, 400 or 1,000 ppm TIBA;

b) Three weeks cold and single sprays of 1,000 ppm IAA, 400 ppm TIBA or 400 ppm GAk;

c) Five weeks cold and single sprays of 400 or 1,000 ppm GAk;

d) Growth regulators applied as a foliar spray rather than with a micropipette;

e) More frequent GAk applications rather than one large application;

f) Three applications of 900 ppm GAk applied at three day intervals in combination with two weeks cold or no cold;

g) Four weeks of cold;

h) Two applications of 200 ppm TIBA plus a single spray of 1,000 ppm GA, and two applications of 200 ppm TIBA plus two weeks cold;

(4) Concentrations of GAk of 270 ppm or less were ineffective in removal of flower bud dormancy;

(5) 900 ppm GAk is effective whether it is applied before or after two weeks of cold;

(6) Foliar sprays of 9 or more ppm NAA can inhibit the rate of flower bud development;

(7) Time of taking data in a flower bud development study of azaleas influences the results.

Bioassays of chromatographed extracts indicate that:

(1) An inhibitor of wheat coleoptile section growth in the Rf 0.5 - 0.6 region decreased after three or more weeks of cold storage;

(2) The inhibitor was not present in vegetative buds;

(3) A promoter of wheat coleoptile section growth in the Rf 0.3 - 0.4 region appeared if the plant had received four weeks of cold storage and three GAk sprays;

(4) The promoter was not present in vegetative buds.

Further tests indicated that the inhibitor could not be considered to be naringenin, and that the promoter could not be considered to be IAA, GA or GAk.

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